REMARKS

With this Amendment, claims 1 and 12 are amended and claims 23 and 24 are canceled without prejudice to further prosecution. Therefore, claims 1-22 are pending with entry of this amendment. Claims 1 and 12 have been amended to recite "wherein said phospholipid mixture comprises a neutral lipid." Support for this amendment can be found in the specification, for example, on page 7, lines 25-28. Claims 1 and 12 have also been amended to recite "said discrete, detectable spots are separately quantifiable." Support for this amendment can be found in the specification, for example, on page 12, lines 18-26, Figure 1, and Figure 2. Therefore, no new matter has been introduced with this Amendment.

Claims 1-6, 12-17 and 23-24 stand rejected under 35 U.S.C. §102(b) as allegedly anticipated by Korte *et al.* and Entezami *et al.* Claims 7-8 and 18-19 stand rejected under U.S.C. §103(a) as allegedly being obvious over either Korte *et al.* or Entezami *et al.* in view of Schmitz *et al.* Claims 10-11 and 21-22 stand rejected under 35 U.S.C. §103(a) as allegedly being obvious over either Korte *et al.* or Entezami *et al.* in view of White *et al.* The rejections will be addressed in the order they were raised in the Office Action.

Rejections under 35 U.S.C. § 102(b)

Claims 1-6, 12-17 and 23-24 stand rejected under 35 U.S.C. § 102(b) as allegedly anticipated by Korte *et al.* (hereinafter referred to as "Korte") and Entezami *et al.* (hereinafter referred to as "Entezami"). The Examiner states that both Korte and Entezami disclose methods of separating phospholipids on a TLC plate into identifiable spots that can be individually detected. The Examiner explains that while the peaks are not as clearly defined as in Applicants invention, the peaks can be identified and assigned a particular phospholipid. In addition, the Examiner states that the Applicants have failed to define the required degree of separation between the peaks.

Applicants agree with the Examiner's statement that the separation achieved by Korte and Entezami is not as defined as the separation achieved by

Applicants' disclosed methods. Applicants respectfully assert, however, that the separation achieved by Korte and Entezami is not sufficient to ***separately quantify*** the individual phospholipids in the mixtures. Therefore, Applicants submit that both Korte and Entezami fail to expressly or inherently describe the element of claims 1 and 12 reciting "said discrete, detectable spots are ***separately quantifiable***."

In Fig. 3, Korte presents a radiometric scan of a TLC plate containing overlapping peaks for phosphatidylserine (PS), phosphatidylinositol (PI) and phosphatidylethanolamine (PE). The overlapping peaks correspond to analyte spots on the TLC plate. It is well known in the art that peaks that are overlapping are not separately quantifiable because overlapping peaks contain a mixture of phospholipid species. Because the peaks in the Korte radiometric scan are overlapping, the peaks contain a mixture of phospholipids. Therefore, the TLC analyte spots from which the peaks are derived contain a mixture of phospholipids and are not separately quantifiable.

For example, measurement of the area under the PS peak in the Korte radiometric scan would not provide an accurate determination of the actual amount of PS because the area is artificially increased by the presence of PI. Therefore, the amount of PS determined from the peak area will be greater than the actual amount of PS present. For the same reason, the amount of PI and PE also cannot be accurately determined from the Korte radiometric scan. Therefore, the analyte spots for PS, PI and PE are not separately quantifiable.

Similarly, Entezami presents a densitometric chromatogram of a TLC plate containing several overlapping peaks, including phosphatidylserine (PS) and phosphatidylcholine (PC) in Fig. 1. The overlapping peaks represent mixtures of phospholipid species. Therefore, the amount of PS and PC cannot be determined accurately by measuring the area under the PS and PC peaks. Because the amount of PS and PC cannot be accurately determined, the PS and PC analyte spots cannot be said to be separately quantifiable.

In contrast, the methods of the presently claimed invention provide non-overlapping peaks for phospholipids wherein each peak contains only a single species of

phospholipid (see **FIG. 2**). Because the peaks are non-overlapping, measurement of the area under each peak provides an accurate determination of the amount of each phospholipid. Therefore, the analyte spots for each phospholipid is separately quantifiable.

Applicants note that both Korte and Entezami provide radiometrically developed TLC plates showing phospholipid spots that do not appear to overlap. However, quantitation of the TLC plates reveal that these apparently non-overlapping spots actually contain a mixture of phospholipids and are, therefore, not separately quantifiable. For example, in Fig. 3 of Korte, the band representing PS on the developed TLC plate appears to be a single, non-overlapping spot. However, the radiometric scan reveals that the PS spot actually contains a mixture of PS and PI. Similarly, in Entezami, the spot on the developed TLC plate representing PC appears to be non-overlapping. However, the densitometric scan reveals that the PC spot contains a mixture of PC and PS. Therefore, simple visualization of spots on the TLC plate is not always sufficient to determine whether an analyte spot on a TLC plate is separately quantifiable.

Thus, Applicants respectfully submit that the phrase "said discrete, detectable spots are separately quantifiable" sufficiently defines the required degree of separation because one of skill would immediately recognize that a spot must contain a single phospholipid species to be separately quantifiable. This recognition is based not only on common knowledge in the art (as discussed above), but also Applicants' teachings in the specification. For example, Applicants teach that

> The most important aspect of quantitation is the degree of separation and tightness of the analyte spots following resolution. Analyte spots that are streaked or smeared and those that are overlapping into adjacent analytes cannot be accurately quantified [see page 12, lines 18-20].

From the above passage, it is clear that non-overlapping analyte spots containing a single phospholipid species are required for an analyte spot to be separately quantifiable.

In summary, both Korte and Entezami fail to resolve all the phospholipids in a mixture into discrete, detectable spots that are separately quantifiable. Therefore,

Applicants assert that both Korte and Entezami fail to expressly or inherently describe the element of claims 1 and 12 reciting "said discrete, detectable spots are separately quantifiable." Thus, Applicants request the withdrawal of the rejections under §102(b).

Rejections under 35 U.S.C. § 103(a)

Claims 7-8 and 18-19 stand rejected under U.S.C. §103(a) as allegedly being obvious over either Korte or Entezami in view of Schmitz *et al.* (hereinafter referred to as "Schmitz"). In addition, claims 10-11 and 21-22 stand rejected under 35 U.S.C. §103(a) as allegedly being obvious over either Korte or Entezami in view of White *et al.* (hereinafter referred to as "White").

Rejection of Claims 7-8 and 18-19 under U.S.C. §103(a) Over Either Korte or Entezami in View of Schmitz

The Examiner states that both Korte and Entezami teach the separation of phospholipids on a TLC plate into identifiable spots that can be individually detected but fail to teach the use of an elution solvent containing chloroform , methanol, acetic acid and an aqueous solution of potassium chloride. The Examiner asserts that Schmitz teaches the separation of phospholipids on a TLC plate using an elution solvent containing chloroform, methanol, acetic acid and an aqueous solution of potassium chloride. The Examiner concludes that it would have been obvious to one of skill in the art to use the elution solvent of Schmitz in the TLC methods disclosed by Korte and Entezami. The Examiner states that the motivation to combine the references is found in Schmitz's teaching that the elution solvent effectively separates several different types of phospholipids, which is equivalent in function to the elution solvents disclosed in Korte and Entezami. Applicants respectfully traverse the rejection.

Applicants respectfully assert that Schmitz does not provide the motivation to combine the references because Schmitz teaches away from Applicants' invention as presently claimed. Applicants have amended claims 1 and 12 to recite "wherein said phospholipid mixture comprises a neutral lipid." Applicants' claimed method is taught in the specification, for example, on page 7, lines 25-28, which states:

> Neutral lipids do not separate from the elution solvent of the present invention and migrate out of the mixture with the solvent front (the leading edge of the solvent as it migrates up the chromatography plate). Consequently, ***the presence of neutral lipids*** in tissue samples ***does not interfere*** with phospholipid detection quantitation.

In contrast, Schmitz teaches that neutral lipids must be removed to separate phospholipids on a TLC plate using an elution solvent containing chloroform, methanol, acetic acid and an aqueous solution of potassium chloride. For example, Schmitz states:

> In our experience, one-dimensional separation of neutral lipids and phospholipids on the same plate can cause contamination of the separate spots by other lipids. In addition, there are calibration problems due to large differences in concentration between individual lipid classes . . . . We conclude, therefore, that the quantitative analysis of neutral and phospholipids in natural mixtures using one-dimensional HPTLC should be done on separate plates to reduce overlapping of lipid compounds [see page 77, lines 20-29, emphasis added].

Thus, Schmitz teaches that the use of an elution solvent containing chloroform, methanol, acetic acid and an aqueous solution of potassium chloride results in overlapping TLC spots containing phospholipid mixtures.

Therefore, after examining Schmitz, one of skill in the art would not be motivated to use an elution solvent containing chloroform, methanol, acetic acid and an aqueous solution of potassium chloride to separate phospholipids on a TLC plate in the presence of neutral lipids, as taught by Applicants. Because Schmitz teaches away from Applicants claimed invention, Applicants assert that it is improper for the Examiner to combine Schmitz with Korte and Entezami (see MPEP § 2145, *In re Grasseli*, 218 USPQ 769, 779 (Fed. Cir. 1983)).

In addition, Applicants assert that the combination of Korte or Entazami in view of Schmitz fails to teach all of the elements of the claimed invention. As described above, Korte and Entezami do not disclose a method of separating a mixture of phospholipids on a TLC plate into discrete, detectable spots that are separately

quantifiable. In addition, Schmitz fails to teach the use of a TLC plate to separate a phospholipid mixture where neutral lipids are present. Therefore, the combination of Korte or Entezami in view of Schmitz fails to teach Applicants claimed method of separating a mixture of phospholipids on a TLC plate into discrete, detectable spots that are separately quantifiable where neutral lipids are present.

In summary, Applicants believe that the claims as previously amended are not obvious over Korte or Entezami in view of Schmitz because Schmitz teaches away from Applicants' claimed invention and the combination fails to teach or suggest all the claimed elements.

Rejection of Claims 7-8 and 18-19 under U.S.C. §103(a) Over Either Korte or Entezami in View of White

The Examiner asserts that White teaches a method for separating phospholipids using TLC and detecting the phospholipid by staining with primulin followed by exposure to ultraviolet light. The Examiner asserts that it would be obvious to one of skill to detect, using the methods of White, the phospholipids separated by the methods of Korte and Entazami. Applicants note that the Examiner has withdrawn the rejection of claims 1-6, 12-17 and 23-24 as allegedly anticipated by White. Therefore, Applicants understand that White is being cited in the current obviousness rejection only for the disclosed detection methods and not the disclosed multiple one-dimensional TLC separation methods.

Applicants respectfully assert that the combination of Korte or Entezami in view of White fails to teach all of the elements of the claimed invention. As discussed above, Korte and Entezami do not teach or suggest a method of separating a mixture of phospholipids on a TLC plate into "discrete, detectable spots that are separately quantifiable." In addition, White fails to teach the use of a single TLC migration to separate all the phospholipids from a phospholipid mixture. Therefore, the combination of Korte or Entezami in view of White fails to teach Applicants claimed method of separating a mixture of phospholipids by allowing the phospholipids to migrate up a TLC

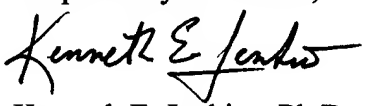
plate until the phospholipids are resolved into discrete, detectable spots that are separately quantifiable.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 415-576-0200.

Respectfully submitted,



Kenneth E. Jenkins, Ph.D.
Reg. No. 51,846

TOWNSEND and TOWNSEND and CREW LLP
Two Embarcadero Center, 8th Floor
San Francisco, California 94111-3834
Tel: 415-576-0200
Fax: 415-576-0300
KEJ:kej
SF 1458809 v1